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Two new chromosome races of the *Agrodiaetus altivagans-wagneri* complex

(Lepidoptera, Lycaenidae)

by

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Summary: The karyotypes of two populations of the genus *Agrodiaetus* were investigated: *A. altivagans altivagans* FORSTER, 1956 (n = 21; Turkey, Gümüshane province) and *A. altivagans vaspurakani* subspec. nov. (n = 22; Turkey, Van province). *A. wagneri iphiactis* subspec. nov. (n = 18) is described from specimens collected by H. DE LESSE near Erzincan, Turkey. The taxonomic position of the investigated taxa is discussed.

Zusammenfassung: Die Karyotypen zweier Populationen der Gattung *Agrodiaetus* aus der Türkei wurden untersucht: *A. altivagans altivagans* FORSTER, 1956 (n = 21; Turkey, Gümüshane) und *A. altivagans vaspurakani* subspec. nov. (n = 22; Turkey, Van). *A. wagneri iphiactis* subspec. nov. (n = 18) wird nach Faltern beschrieben, die von H. DE LESSE in Erzincan, Türkei gesammelt wurden. Die taxonomische Stellung der untersuchten Taxa wird diskutiert.

Introduction

The karyotypes of different populations related to *Agrodiaetus altivagans* FORSTER, 1956 were investigated first by famous French entomologist HUBERT DE LESSE (1962). DE LESSE found a great karyotype variability in this complex, with chromosome numbers ranging from n = 17 in Sanandadj (W Iran) to n = 23 in Dogubayazit (E. Turkey). One of these chromosome races was described by DE LESSE (1963) as *A. altivagans ectabanensis* (n = 18, NW Iran). However, the taxonomical interpretation of these data was difficult until the karyotype of the nomynotypical population of *A. altivagans* from Armenia (n = 21) was studied (LUKHTANOV & DANTCHENKO, 2002a).

In this paper we present the results of our recent investigation of two additional Turkish populations close to *A. altivagans*. One of them was found in the southern part of Gümüshane province (50–60 km N of Erzincan). Apparently it is the most western known spot of the range of *A. altivagans*. Another population originates from Lake Van in SE. Turkey, from which region numerous endemic taxa of Lycaenidae were described (HESSELBARTH, OORSCHOT & WAGENER, 1995; LUKHTANOV & DANTCHENKO, 2002b).

We analyzed also published (DE LESSE, 1962) and unpublished data of DE LESSE who discovered another chromosome race of „*A. altivagans*" (n = 18) in the same region north and west of Erzincan (Turkey). The last chromosome form is probably not conspecific with *A. altivagans* because these races have parapatric (or may be even sympatric) ranges.

Materials and methods

Population samples of studied taxa were collected in Turkey in 2001. Fresh adult males were used to investigate the karyotypes. After capturing a butterfly in the field, it was placed into a

glassine envelope for 1–2 hours to keep it alive until we are ready to process it. Then the butterfly was killed by pinching it firmly on the thorax. Immediately after killing it, the testes were removed from the abdomen and placed into a small 0.5 ml vial with a freshly prepared Carnoy fixative (ethanol and glacial acetic acid, 3 1). Then each wing was carefully removed from the body. The wingless body was placed into a plastic, 2 ml vial with pure 100% ethanol. Each vial with ethanol has already been numbered. This ID-number was also used to label a vial with a Carnoy fixative and a glassine envelope in which to preserve the wings. Thus, each specimen was individually fixed. After the fixation we had three components collected for each butterfly, each of which was identified by a common ID-number: (a) a vial containing the butterfly testes (for karyotype analysis), (b) a vial containing the butterfly wingless body (for DNA analysis) and (c) a glassine envelope containing the wings.
Testes were stored in the fixative for 1–12 months at +4 °C. Then the gonads were stained in 2% acetic orcein for 30–60 days at +18–20 °C. Different stages of male meiosis were examined in a light microscope Amplival, Carl Zeiss. We have used an original two-phase method of chromosome analysis (LUKHTANOV & DANTCHENKO, 2002a).
Negatives and photographs of the studied chromosome preparations are kept in the Department of Entomology of the University of St. Petersburg, Russia. The set specimens of the donor butterflies (the butterfly wingless bodies in ethanol and wings in glassine envelopes) are kept in the DNA and Tissues Collection of Museum of Comparative Zoology (Harvard University, Cambridge, MA, USA).
Females were not included in the type-series and in the descriptions of new taxa because we failed females in copula with karyotyped males.

Abbreviations:
MI – first methaphase of meiosis
MII – second metaphase of meiosis
MCZH – Museum of Comparative Zoology (Harvard University, Cambridge, MA, USA)
MNHN Muséum National d'Histoire Naturelle (Paris, France).
n – haploid chromosome number

Descriptions of chromosome races

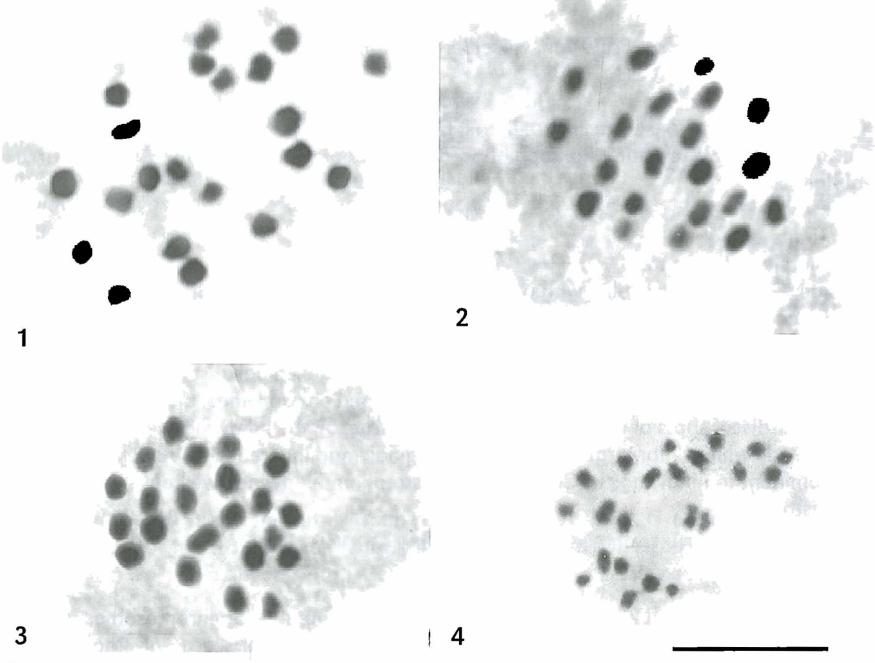
1. *Agrodiaetus altivagans altivagans* from Gümüshane Province
(col. pl. XXVIII, figs. 1–4)

Material
No VL01L111, n = 21, Turkey, Prov. Gümüshane, 25 km S Torul, 40°20'N, 39°18'E; 1400 m, 14.VII.2001, A. DANTCHENKO & V. LUKHTANOV leg., in MCZH.
No VL01L112, n = 21, Turkey, Prov. Gümüshane, 25 km S Torul, 40°20'N, 39°18'E; 1400 m, 14.VII.2001, A. DANTCHENKO & V. LUKHTANOV leg., in MCZH.
No VL01L113, n = 21, Turkey, Prov. Gümüshane, 25 km S Torul, 40°20'N, 39°18'E; 1400 m, 14.VII.2001, A. DANTCHENKO & V. LUKHTANOV leg., in MCZH.
No VL01L132, n 21, Turkey, Prov. Gümüshane, Dilekyulu, 40°24'N; 39°16'E; 1300 m, 15.VII.2001, A. DANTCHENKO & V. LUKHTANOV leg., in MCZH.

Karyotype (figs 1, 2)

The haploid chromosome number in *A. altivagans altivagans* from Gümüshane Province was precisely determined as n = 21. This count is based on study of 15 MI cells in the specimen No VL01L111; of 7 MI, 4 MII and 7 prometaphase cells in the specimen No VL01L112; of 14 MI cells in the specimen No VL01L113 and of 15 MI cells in the specimen No VL01L132. In MI all bivalents form a gradient series. The karyotype shows no extraordinary large or small chromosomes.

The same chromosome number (n = 21) was found by DE LESSE in specimens from Mirgemir Dagh and Ararat in E. Turkey (DE LESSE, 1962) and by us in Armenia (LUKHTANOV & DANTCHENKO, 2002a). Interestingly, the mtDNA sequences of COI and COII are very similar in the specimens from Armenia and Gümüshane, Turkey (KANDUL et al., in press). Thus, this chromosome race (n = 21) of *A. altivagans* is widely distributed in NE. Turkey and Armenia.



Figs 1–4: Karyotypes of *Agrodiaetus altivagans*.
Figs 1, 2: *Agrodiaetus altivagans altivagans*, No VL01L112, n = 21, Turkey, Prov. Gümüshane, 25 km S Torul, 40°20'N, 39°18'E; 1400 m, 14.VII.2001, A. DANTCHENKO & V. LUKHTANOV leg., in MCZH. 1 – prometaphase of meiosis, 2 – MI.
Figs 3, 4: *Agrodiaetus altivagans vaspurakani* subspec. nov.
Fig. 3: Paratype, VL01L354, n = 22, MI, Turkey, Prov. Van, Güseldere Geçidi, 2700 m, 24.VII.2001, A. DANTCHENKO & V. LUKHTANOV leg., in MCZH.
Fig. 4: Holotype, No VL01L222, n = 22, MII, Turkey, Prov. Van, 34 km N Çatak, 22.VII.2001, A. DANTCHENKO & V. LUKHTANOV leg., in MCZH.
scale bar = 10µm

2. *Agrodiaetus altivagans* ***vaspurakani*** **subspec. nov.**
(col. pl. XXVIII, figs. 5–8)

Material
Holotype ♂: No VL01L222, n 22, Turkey, Prov. Van, 34 km N Çatak, 22.VII.2001, A. Dantchenko & V. Lukhtanov leg., in MCZH.
Paratypes: ♂VL01L354, n = 22, Turkey, Prov. Van, Güseldere Geçidi, 2700 m, 24.VII.2001, A. Dantchenko & V. Lukhtanov leg., in MCZH.
♂VL01L368, n = 22, Turkey, Prov. Van, Güseldere Geçidi, 2700 m, 24.VII.2001, A. Dantchenko & V. Lukhtanov leg., in MCZH.
♂VL01L369, n = 23, Turkey, Prov. Van, Güseldere Geçidi, 2700 m, 24.VII.2001, A. Dantchenko & V. Lukhtanov leg., in MCZH.
♂VL01L386, n = 22, Turkey, Prov. Van, Güseldere Geçidi, 2700 m, 25.VII.2001, A. Dantchenko & V. Lukhtanov leg., in MCZH.
♂2001-472, n = 22, Turkey, Prov. Van, Güseldere Geçidi, 2700 m, 25.VII.2001, A. Dantchenko & V. Lukhtanov leg., in MCZH.
♂ No 92121, n = 22 and n = 23, Turkey, Prov. Van, Güseldere Geçidi, 2650–2850 m, 4.–5.VIII.1992, leg. H. v. Oorschot, H. v. d. Brink, D. v. d. Poorten & W. De Prins, in the collections of the Institute of Systematic and Population Biology (Zoological Museum), Amsterdam, the Netherlands.

Description
♂. Forewing length 17 mm.
Upperside: Ground color is blue with a light violet tint, veins darkened distally, more on hindwings. Discoidal, submarginal and antemarginal marking completely absent. Forewing with androconial scales. Inner part of fringes is dark gray, outer part is white.
Underside: Ground color is gray with a light brownish tint. Bluish basal suffusion is not strong but clearly prominent. Discoidal black spot well developed on forewings, on hindwings almost invisible. Postdiscal spots of the forewings relatively large, on the hindwings very small, clearly encircled with white. Submarginal and antemarginal marking reduced, faintly discernible, more prominent in hindwings in Cu1–2A. White streak on hindwings well developed, not enlarged distally.
♀ unknown.

Karyotype (figs 3, 4)
In the holotype (No VL01L222) in 7 studied MII cells, the number of chromosomes was precisely determined as n = 22. The karyotype shows no extraordinary large or small chromosomes. All of them form a gradient series. In the paratype specimens VL01L354, VL01L368, VL01L386 and 2001-472, the karyotype n = 22 was found in all studied cells. This count is based on 80 MI, 7 MII and 5 diakinetic cells. In the specimen VL01L369, the chromosome number n = 23 was observed in 11 MII cells; no MI cells were found in this specimen. In No 92121 which were previously determined as „*A. firdussi pseudactis*" (Lukhtanov et al., 1998) in 3 studied MI cells the same chromosome number, n = 22 was found. Three other MI cells of the specimen No 92121 showed the chromosome number n = 23 (Lukhtanov et al., 1998).

Ecology
The new taxon was found in highland steppe-like biotopes at about 1900–2600 m, on slopes covered with a rich xerophilous vegetation. The butterflies were flying together with *A. turcicolus* KOÇAK, 1977, *A. pierceae* LUKHTANOV & DANTCHENKO, 2002, *A. vanensis* DE LESSE, 1957, *A. baytopi* DE LESSE, 1959 and *A. haigi* DANTCHENKO & LUKHTANOV, 2002.

Differencial diagnosis and discussion
According to the phenotype, *A. altivagans vaspurakani* is most similar to *A. wagneri wagneri* FORSTER, 1956 (n = 16), but has a very different karyotype. *A. wagneri wagneri* has n = 16 (DE LESSE, 1962), while n = 22–23 was found in *A. altivagans vaspurakani*. With this chromosome number (n = 22–23), the new taxon can be also distinguished from the allopatric chromosome races *A. altivagans altivagans* (n = 21) and *A. altivagans ectabanensis* (n = 18). Molecular data show that *A. altivagans altivagans* and *A. altivagans vaspurakani* belong to two different evolutionary lineages in the genus *Agrodiaetus*. *A. altivagans altivagans* is closely related to *A. damocles* (HERRICH-SCHÄFFER, [1844]), while *A. altivagans vaspurakani* is related to the „brown" species *A. mithridates* (STAUDINGER, 1878) (KANDUL et al., in press). Therefore, the taxon *vaspurakani* seems to be a good species in fact. Additional investigations will clarify this complicated situation.
We can not exclude that the population from Dogubuyazit, which is geographically close to the Lake Van region and has varying chromosome numbers n = 21–23, belongs also to *A. altivagans vaspurakani*. However, this supposition should be tested by studying the DNA sequences.

Etymology
Vaspurakan is an old historical name of the geographical region around Lake Van, where the new taxon was collected.

3. *Agrodiaetus wagneri* ***iphiactis*** **subspec. nov.**
(col. pl. XXVIII, figs. 9–11)

Material
Holotype ♂: n = 18, Erzincan (Turq.), 47 km W, 2000 m, 21.VII.1959, H. DE LESSE leg., in MNHN.
Paratypes: 3 ♂♂, n = 18, Erzincan (Turq.), 47 km W, 2000 m, 21.VII.1959, H. DE LESSE leg., in MNHN. 1 ♂, n = 18, Erzincan (Turq.), 25 km N, 2600 m, H. DE LESSE leg., in MNHN.

Description
Upperside: Ground color is blue, veins strongly darkened distally, more on hindwings. Discoidal, submarginal and antemarginal marking completely absent. Forewing with androconial scales. The inner part of the fringes is dark gray, outer part is white.
Underside: Ground color is gray with a light brownish tint. Bluish basal suffusion is not strong but clearly prominent. Discoidal black spot well developed on forewings, on hindwings almost invisible. Postdiscal spots of the forewings relatively large, on the hindwings very small, clearly encircled with white. Submarginal and antemarginal marking reduced, faintly discernible, more prominent in hindwings in Cu1–2A. White streak on hindwings well developed, but not contrasting, not enlarged distally.
♀ unknown.

Karyotype
The specimens designated here as holotype and paratypes were determined by H. DE LESSE (1962) as „*A. altivagans altivagans*„. H. DE LESSE investigated their karyotype and determined the chromosome number in all studied cells (MI and MII) as n = 18. No variability of chromosome number was found.

Differential diagnosis
This taxon can be differentiated by its karyotype n = 18 from *A. wagneri wagneri* (n = 16) and from *A. altivagans altivagans* (n = 21). The same chromosome number (n = 18) was found in *A. altivagans ectabanensis* which has an isolated distribution in Iran. However, *A. altivagans ectabanensis* can be easily distinguished by its phenotype (DE LESSE, 1962, 1963). It is most likely that *A. altivagans ectabanensis* and *A. wagneri iphiactis* belong to two different, not closely related evolutionary lineages in the genus *Agrodiaetus*.

Discussion
According to the phenotype of butterflies and to the chromosome number, the form n = 18 from Erzincan region is most similar to *A. wagneri* while the form n = 21 from the same region is similar or even identical to *A. altivagans*. Therefore the attributation of the race n = 18 to *A. wagneri* seems to be the most realistic taxonomic hypothesis. DE LESSE supposed that the chromosome race n = 18 is allopatric in distribution to the race n = 21. Therefore he considered them as conspecific populations of *A. altivagans*. We found the race n = 21 in immediate proximity to the race n = 18. Both of them fly 20–60 km to the north of Erzincan. Thus, they are at least parapatric in distribution. According to the field observation and to the collecting data, *A. altivagans* and *A. wagneri* fly together in this region (HESSELBARTH, OORSCHOT & WAGENER, 1995). The last indication was so far not confirmed by karyological data. However, it seems to be very probable that *A. altivagans* and *A. wagneri iphiactis* have sympatric ranges. These distributional data do not agree with the statement about the conspecifity of chromosome races n = 18 and n = 21.

A. erzindjanensis CARBONELL, 2002, a taxon apparently closely related to *A. altivagans*, was recently described from Spikör Geçidi, prov. Erzincan, Turkey. Unfortunately, the karyotype of *A. erzindjanensis* was not investigated. Therefore, its status remains uncertain (i. e. species incertae sedis). The probability that *A. wagneri iphiactis* and *A. erzindjanensis* are conspecific seems to be low because these taxa have different number of antennal segments (36 and 31–33 respectively) (see CARBONELL, 2002 and col. pl. XXVIII, fig. 11 in this paper). We can not exclude that *A. erzindjanensis* is conspecific with *A. altivagans* (n = 21).

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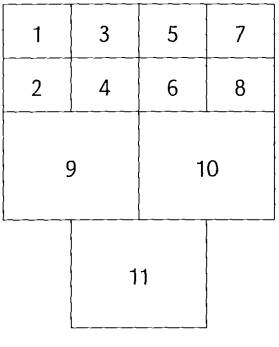
Explanation of colour plate XXVIII (p. 481):

Figs 1-4: *Agrodiaetus altivagans altivagans* FORSTER, 1956
No VL01L111, n = 21, Turkey, Prov. Gümüshane, 25 km S Torul, 40°20'N, 39°18'E; 1400 m, 14.VII.2001, A. DANTCHENKO & V. LUKHTANOV leg., in MCZH.
Figs 5-8: *Agrodiaetus altivagans vaspurakani* subspec. nov.
Holotype ♂: No VL01L222, n = 22, Turkey, Prov. Van, 34 km N Çatak, 22.VII.2001, A. DANCHENKO & V. LUKHTANOV leg., in MCZH.
Figs 9-11: *Agrodiaetus wagneri iphiactis* subspec. nov.
Holotype ♂: n = 18, Erzincan (Turq.), 47 km W, 2000 m, 21.VII. 1959, H. DE LESSE leg., in MNHN.
9 - upperside, 10 - underside, 11 - labels.



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Colour plate XXVIII

LUKHTANOV, V. A. & A. V. DANTCHENKO: Two new chromosome races of the *Agrodiaetus alti-vagans-wagneri* complex (Lepidoptera, Lycaenidae). - Atalanta **34** (3/4): 421-428.

Figs 1-4: *Agrodiaetus altivagans altivagans* FORSTER, 1956
No VL01L111, n = 21, Turkey, Prov. Gümüshane, 25 km S Torul, 40°20'N, 39°18'E; 1400 m, 14.VII.2001, A. DANTCHENKO & V. LUKHTANOV leg., in MCZH.
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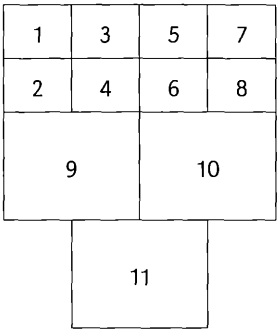
9 - upperside, 10 - underside, 11 - labels.



Colour plate XXVIII